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13. ABSTRACT *(Maximum 200 words)*

We have elucidated the mechanisms of hydrolysis of phosphate diesters by acid and metal ion catalysis. In addition a new catalyst carrier (microgonotropens) to DNA and RNA have been invented, synthesized (patented) and the reactions with DNA studied. Such information is particularly important to those interested in the catalysis of hydrolysis of DNA and RNA.

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FINAL REPORT

Grant # : ONR N00014-90-J-4132

PRINCIPAL INVESTIGATOR: THOMS C. BRUICE
INSTITUTION: University of California At Santa Barbara

GRANT TITLE: The Dual and Simultaneous Roles of Nucleophile delivery and Assistance to Leaving Group Departure by Metal Ions in Phosphate Transfer From Phosphate Diesters

AWARD PERIOD: 14 Sept 1990 to 30 April 1998

OBJECTIVE: To elucidate the mechanisms of catalysis of hydrolysis of phosphate diestrs, to invent systems which can be used as (among other things) cariers of catalytic entities to DNA for their hydrolysis.

APPROACH ACCOMPLISHMENTS & CONCLUSIONS: We have published 35 manuscripts supported completely or partially by this ONR grant. These publications fall into three categories: A) catalysis of hydrolysis of organic phosphate diesters; B) an invented class (Microgonotropens) of DNA minor to major groove binding agents with planed use as catalyst carriers for the hydrolysis of DNA. Initial publications dealing with the first synthesis of DNG and RNG (the initiation of the research that would be further supported by ONR N00014-96-1-0123); and C) subjects dealt with by computational chemistry. Concerning "C", this grant, at an early time, supported the growth of hardware in the P.I.'s computational facility and this has allowed solution of computational problems that do and do not fall into categories "A" and "B".

Catalysis of the hydrolysis of phosphate diesters by various functional groups were studied as intramolecular reactions with single turnovers. One and two functional group catalysis as well as with one, two, and three metal ion catalysis were investigated. Systems with two neighboring pyridine amine nitrogens establish nucleophilic attack of one of the nitrogen upon phosphate, the absence of general-acid catalysis but the presence of metal ions assistance to the departure of the leaving group.[1] In the latter rate enhancements of 10^3 M were observed. Several systems involving nucleophilic catalysis by a $-CO_2^-$ concerted with general acid catalysis by a $-CO_2H$ group were investigatd.[2,3,4] The most effective exhibited rate enhancements of 10^9 M.

Dinucleotides were designed and synthesized such that metal ions could be ligated near the phosphate moiety. In the first step of hydrolysis

these "ribonucleotides" undergo intramolecular transesterification by specific HO^- ionization of the ribose 2'-OH which is catalyzed by Zn^{2+} (10^5-fold), Mg^{2+} (10^3), Cu^{2+} (10^5), and La^{3+} (10^9-fold). Whereas in the Zn^{2+} complex the metal ion owes it's catalysis to the neutralization of the negative charge on the O-(PO_2^-)-O- the more efficient La^{3+} is capable of also ligating the leaving oxyanion. Reasons for the catalytic efficiency of La^{3+} are treated.[5,6]

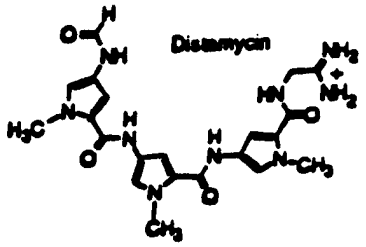
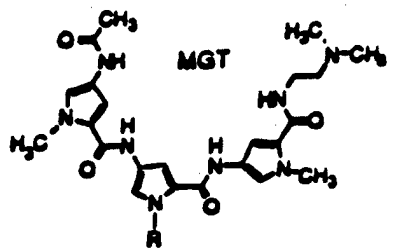
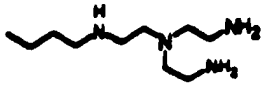
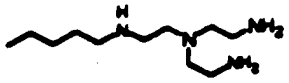
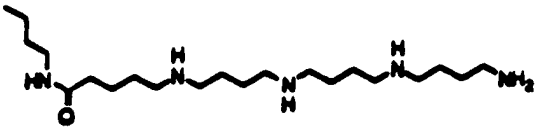
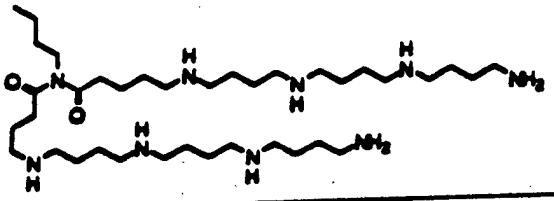
Phosphodiesters and phosphonate esters have like rates of hydrolysis if the leaving groups are the same. Using a phosphonate linkage it was possible to design and synthesize an ester that ligates two La^{3+} ions. Hydroxide ion is ligated by one metal ion and held in such a fashion that it is inline with the leaving group and adjacent to the phosphate phosphorous. This HO^- is in perfect position to carry out a displacement reaction at the phosphate linkage. The same La^{3+} which ligates the HO^- also interacts with one of the two -(PO_2^-)- oxygens. The second La^{3+} interacts with the other -(PO_2^-)- oxygen as well as the leaving group. All possible interactions for catalysis are fulfilled. The rate enhancement is 10^{13}-fold.[7,8] We have been invited to prepare a review of our work and it should appear in March 1999.[9]

The structures of our synthetic Microgonotropens are provided in Table I..[10] Studies of the synthesis,[11,12,16,18,20] foot-printing[12,16], determination of equilibrium constants for Microgonotropens binding to DNA sequences[13] and the determination of structures of microgonotropen·DNA complexe[14,15,17,19] have been carried out. Our purpose in the development of the microgonotropen technology was to arrive at synthetic materials with DNA nuclease activity. Microgonotropens have attracted attention from biologists as well. A key component of gene regulation is the binding of transcription factors (TFs) to promoter elements containing their consensus DNA binding site. Targeting of E2F1-DNA complexes with microgonotropens has been carried out (Table 1). The most effective in inhibiting complex formation between E2F1 and the dihydrofolate reductase promoter is MGT-6a which exhibited 50% inhibition of complex formation at 0.00085 μM.[1] Our concept of the microgonotropen has been patented and is being included into the structures of Peter Dervan.

Peptide nucleic acids (PNA) are tight binders to DNA and as such of interest to us. Several Molecular Mechanics studies of the structures of Polyamide nucleic Acid DNA Duplexes and Triplexes have been published.[21,22]

The subject of ONR N00014-96-1-0123 is the synthesis and study of DNA and RNA mimics in which the negatively charged phosphate diester linkages of DNA and RNA [-O-(PO_2^-)-0-] are replaced by positively charged linkers as the guanido linker [-NH-(C=NH_2^+)-NH-] in DNG and

Table 1. Association constants for MGT compounds with d(GGCGA$_3$T$_3$GGCGG)/ d(CCGCCA$_3$T$_3$GCGCC) (in H_2O, 10 mM phosphate buffer, pH 7.0/10 mM NaCl at 35°C)

Compound	log(K_1)	log(K_2)	log(K_1K_2)
 Distamycin	7.6	8.4	16
 MGT			
2 R= $-CH_3$	6.8	6.2	13
1 R= 	8.4	8.0	16.4
5a R=	8.5	8.9	17.4
6a R= 	9.2	9.2	18.4
6b R= 	8.9	9.0	17.9
7 R= 	7.6	9.2	16.7
8 R= 	9.3	7.9	17.3

RNG. The first DNG and RNG studies were carried out with the financial assistance from the present grant..[22,23,24,25,26]

Computational studies which have benefited by use of the computer allowed in the budget of the present grant[27 to 38] carry the acknowledgment "We thank the ONR for support of our computational facility."

SIGNIFICANCE: We have elucidated the mechanisms of hydrolysis of phosphate diesters by acid and metal ion catalysis. In addition a new catalyst carrier (microgonotropens) to DNA and RNA have been invented, synthesized and the reactions with DNA studied. Such information is particularly important to those interested in the catalysis of hydrolysis of DNA and RNA.

PATENT:
T. C. Bruice, K. A. Browne, and G-X. He. TRIHETEROCYCLIC PEPTIDES CAPABLE OF BINDING THE MINOR AND MAJOR GROOVES OF DNA, U. S. Patentt Number 5,698,674. Allowed 12/16/97

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